DRAFT RSET ISSUE PAPER #20 – Testing Protocols For In-Situ Freshwater Bioaccumulation Testing

BIOACCUMULATION SUBCOMMITTEE, D. Kendall (david.r.kendall@usace.army.mil) and T. Michelsen (teresa@avocetconsulting.com), Co-Chairs; September 19, 2004

QUESTION/ISSUE: What are the available in-situ freshwater bioaccumulation testing protocols and what are the known advantages and disadvantages of these protocols?

DISCUSSION:

Background: Consensus-based ASTM protocols have been developed for in-situ caged bivalves that can be used to assess bioaccumulation potential and associated biological effects from contaminated sediments in marine, estuarine and freshwater species (ASTM 2001). Over 30 freshwater studies have been conducted in freshwater environments using these methods and 7 different species. These studies include five Superfund sites; three marine and two freshwater. The main advantage of this approach is the ability to characterize exposure and effects over space and time and under environmentally realistic test conditions. The main disadvantage is the cost, although costs do not increase incrementally with time as in laboratory toxicity or bioaccumulation tests because daily maintenance is not required. Other advantages and disadvantages are summarized in Table 1 (Salazar & Salazar 1998).

Protocols have also been developed for freshwater toxicity tests using species other than bivalves that may be adaptable for in-situ bioaccumulation testing (Burton 2002). The most promising candidate among those protocols is the oligochaete *Lumbriculus variegatus*, although questions have been raised regarding the small tissue mass and this species was not reported in a recent survey of the Lower Columbia River (Waldeck et al 2003). The main advantage of *Lumbriculus* is that methods exist for in-situ testing and it has been used routinely in the laboratory for toxicity testing across the country for many years. The analogous disadvantage in the marine environment would be the polychaete worms *Neanthes arenaceodentata* and *Armandia brevis*. While they too have been used extensively for toxicity testing, they have been used less for bioaccumulation testing because of their relatively small tissue mass. Nevertheless, these species have been used effectively for both toxicity and bioaccumulation testing.

In-situ testing is needed as part of RSET to bridge the gap between traditional laboratory testing and field monitoring and help establish links between bioaccumulation data collected using those methods. Since effects endpoints such as survival, growth, and reproduction have been developed for some bioaccumulation test species, in-situ testing can also help integrate toxicity and bioaccumulation testing. Other advantages include validation of results from laboratory bioaccumulation testing and integration of results from field monitoring, assessment of long-term exposures and associated effects, and the ability to characterize benthic exposure pathways under environmentally realistic conditions. While there are no perfect monitoring tools, bivalves satisfy many of the criteria identified for being a practical in-situ testing organism for bioaccumulation potential and associated biological effects.

<u>Discussion</u>: The Bioaccumulation Subcommittee identified the need to summarize the current state of in-situ freshwater bioaccumulation protocols and options available for the development of new freshwater test protocols and species. The need for using two species for assessing bioaccumulation potential was also discussed. A list of criteria for selecting appropriate species for in-situ bioaccumulation tests was compared with a list of species found in the Lower Columbia River in a recent survey to help develop a candidate list of indicator organisms. In the context of RSET, criteria used for selecting organisms to assess bioaccumulation potential and protect higher trophic level wildlife should be similar to those used for selecting toxicity test organisms. Criteria for selecting candidate freshwater species for testing should be similar to those used for selecting marine species. The selection of a suitable organism is one of the first steps in the preparing a monitoring strategy once the decision to conduct bioaccumulation testing has been made. The importance of this step cannot be overemphasized. Several attributes of both the organism and the study area must be considered. Furthermore, no one organism is best suited for all aquatic ecosystems (Burton 2002, Phillips 1980). This is another reason for using two bioaccumulation test species instead of only one.

Species Selection Criteria
The following criteria for selecting candidate species for in-situ bioaccumulation testing were synthesized from several different sources which included different perspectives (Burton 2002, Phillips 1980, Widdows & Donkin 1992). A test organism should:

a) Accumulate chemicals at concentrations in test sediment without being killed
b) Be sedentary, to represent the study area and minimize caging effects
c) Be abundant, with stable populations in the area for ecological relevance
d) Be sufficiently long-lived to allow the sampling of more than one year-class
e) Be of reasonable size, giving adequate tissue for analysis
f) Be easy to sample and robust enough to survive in the laboratory
g) Be easy to collect, cage, and make bioaccumulation measurements on
h) Have a large toxicological database for pairing exposure and effects endpoints
I) Be easily identified
j) Have standardized protocols available
k) Allow integration of laboratory testing, field monitoring, and in-situ experiments
l) Have a relatively low ability to metabolize accumulated chemicals

Candidate Test Species

Based on the criteria identified above, three groups of organisms were selected as satisfying the criteria and being in the LCR. In order of preference these were 1) bivalves; 2) gastropods; and 3) decapods (crayfish).

Bivalves
Fourteen to 15 species of freshwater bivalves are found in the Lower Columbia River. These include the invasive species *Corbicula fluminea* and four native unionids in the genus *Anodonta*. Although *Anodonta* satisfy many of the criteria and may be more sensitive than Corbicula, they are not recommended for large-scale monitoring and testing because of their declining numbers and uncertain taxonomy. The dichotomy is that while native unionids need to be studied to preserve them, their numbers may be too small to collect in large numbers to support extensive monitoring and assessment. Many more studies have been conducted on *Corbicula* throughout the world, including laboratory bioaccumulation and toxicity tests, field monitoring, and transplant experiments, and they have been found in almost every previous survey conducted on the Lower Columbia River. In addition, a laboratory bioaccumulation test is being proposed for this species as

part of RSET.

Corbicula fluminea is an introduced freshwater bivalve found throughout freshwater environments in the Pacific Northwest in large numbers and therefore is ecologically relevant. Side-by-side bioaccumulation tests have been conducted using *Corbicula fluminea* and *Lumbriculus variegatus* using 28-day laboratory exposures to candidate reference sediments collected in the Willamette River in Oregon (Hart Crowser, 2002). While one of the concerns regarding the use of *Corbicula fluminea* has been the uptake kinetics of this species relative to *Lumbriculus variegatus* and valve closure to avoid exposure in a short-term 28-d exposure, this is not an issue in standard in-situ testing protocols that suggest an exposure period of 60 to 90 days for most species and most chemicals. Bivalves are able to close their valves to avoid exposure in short-term tests and reduce their respiration and filtration rates, which would consequently reduce exposure to sediment-associated contaminants. However, in longer term tests, effects would be manifested in reduced survival and growth rates. This is another reason for pairing exposure and effects endpoints in toxicity and bioaccumulation tests.

Also included in this list are 7 species of fingernail clams. The main advantage of fingernail clams is their small size. This makes them very suitable for laboratory and field testing but their small size is also a disadvantage for bioaccumulation potential for the same reason that *Lumbriculus* has a disadvantage. Nevertheless, they should be considered for bioaccumulation and toxicity testing. Fingernail clams have been shown to be extremely sensitive to ammonia, among other chemicals, and have been used in a number of in-situ toxicity tests. While ammonia is not easily measured in bivalve tissues, fingernail clams should be placed in the category of candidate species for in-situ bioaccumulation testing and laboratory toxicity testing.

Gastropods

Gastropods also have a good potential for freshwater monitoring because they also satisfy many of the criteria for selecting candidate test species. Many species related to those found on the Lower Columbia River have been used in laboratory bioaccumulation and toxicity tests, field monitoring and even transplant experiments. However, they have not been used as extensively as bivalves. Although 35 different species have been reported in the literature, only 14 of those were found in the most recent surveys. Perhaps more importantly, these gastropods are classified as deposit feeders and potentially in more direct contact with sediment. In a weight-of-evidence approach with multiple species they would be a good second choice for laboratory testing, field monitoring, and transplant experiments.

Crayfish

Only one species of freshwater crayfish has been reported in the LCR and it was also found in the most recent Lower Columbia River surveys. *Pacifastacus leniusculus leniusculus* has the potential to be an important species for laboratory testing, field monitoring and transplant experiments. However, since only one species was found and it has been used far less than either bivalves or gastropods we ranked it third in terms of recommended species but as with the gastropods, remains potentially useful, particularly in a weight of evidence approach. Additionally, it represents a different pathway of exposure in that the dietary exposure pathway should dominate, particularly for hydrophobic organic chemicals.

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RECOMMENDATION:

The Bioaccumulation Subcommittee recommends *Corbicula fluminea* as the first choice for in-situ assessments of bioaccumulation potential because it has been used extensively in laboratory testing, field monitoring, and in-situ assessments of both toxicity and bioaccumulation potential. The Bioaccumulation Subcommittee recommends the following tasks to be completed:

1. Compile and evaluate existing data on bioaccumulation by *Corbicula* fluminea and another species to be selected.

2. Conduct additional bioaccumulation testing in the lab and the field using *Corbicula* as projects allow.

3. Conduct additional bioaccumulation testing for other candidate species.

4. Conduct synoptic bioaccumulation tests in the laboratory and in-situ using *Corbicula* and the second candidate test species.

PROPOSED LANGUAGE: None yet available.

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Table 1. Advantages and disadvantages of the in-situ field bioassay by category: transplants, bivalves, bioaccumulation, and growth.

	Transplants	**Bivalves**	**Bioaccumulation**	**Growth — Whole Animal/Tissue**
Advantages	Experimental control Environmental realism Defined exposure period Infinite sampling matrix Repetitive, non-destructive sampling Monitoring individuals Field validation Exposure system Captive biochemical sampling Hypothesis testing Low maintenance	Integrate bioavailable contaminants Bioconcentrate contaminants Easy to collect, cage, measure Large database from field monitoring and lab bioassays Survive sub-optimal conditions Any biochemical measurements possible Sedentary No feeding required Standardized protocols	Concentrations above ambient Integration of contaminants, natural factors, man-made non-toxics Assessments for sediment, overlying water, or porewater Link between exposure and response Link between lab and field Link between bioassays and community structure Long-term exposures –1 yr	Integration of internal biological processes Environmentally significant response Link to population effects Quantifiable dose-response Related to environmental exposures Repetitive, non-destructive measurements Easy for the public to understand No special equipment No specialized training More sensitive than survival Long-term exposures –1 yr
Disadvantages	Effects of transplanting Loss of cages from acts of nature, inadvertent capture by moving vessels, vandalism Cost of collection, sorting, deployment Possible caging effects	Not found in all areas May not be representative of assessment area May not be the most sensitive species May not directly assess community effects May close to avoid exposure in short-term tests	Affected by chemical and natural factors Not all contaminants are accumulated equally Some contaminants may be purged May not always accurately represent effective dose	Affected by chemical and natural factors May not be the most sensitive bioeffect Tissue and shell growth occur at different rates and are affected by different factors May not directly assess community effects